

# LABORATORY NOTEBOOK

MBX-1212

Notebook No.: 6

Assigned to: Lara Madison

Date: \_\_\_\_\_

Use Nalge Cat. No.

6301-1000  
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Design primers for sequencing A1/B1 Fusions

1296..2036 ="phbB"

20  
1296  
240

FB.1

FB.2

FB.3

atgac tcagcgatt gcgtatgtga  
ccggcgcat ggggtggtatc ggaaccacca tttgccagcg gctggccaag ggtggtttc  
gtgtggtggc cgggttgcgc cccaactcgc cgcgcgcga aaagtggctg gacgcagaga  
aggccctggg cttcgatttc attgectcgg aaggcaatgt ggctgactgg gactcgacca  
agaccgcatt cgacaaggte aagtcggagg tcggcgaggt tgatgtgtg atcaacaacg  
ccggtatcac ccgcgacgt gtgttcgca agatgaccg cgcgcactgg gatcggtga  
tcgacaccaa cctgactcgc ctgttcaacg tcaccaagca ggtgatcgac ggcatggccg  
accgtggctg gggccgcacg gtaacatct cgtcggtgaa cgggcagaag ggccagtctg  
gccagaccaa ctactccacc gccaaaggcg gctgcatgg cttcaccatg gcactggcgc  
aggaagtggc gaccaaggcg gtgaccgtca acacggctc tccgggctat atcgccaccg  
acatggctca ggcgatccgc caggacgtgc tcgacaagat gtcgcgacg atcccggtca  
agcgctggg cctgcgggaa gagatcgct cgatctgcg ttggtgtcg tcggaggagt  
ccggtttctc gaccggcgcc gacttctcgc tcaacggcgg cctgcatatg ggctga

Table of Contents

40..1221 ="phbA"

FA.1

FA.2

FA.3

FA.4

a tgactgacgt tgtcatcgta  
tccgcgcgcc acaccgcggg cggaagttt ggcggtcgc tggccaagat ccggcaccg  
gaactgggtg ccgtggtcat caaggccgcg ctggagcgcg ccggcgtaaa gccggagcag  
gtgagcgaag tcactatggg ccagggtgtg accgcgggtt cgggccagaa ccccgacgc  
caggccgcga tcaaggccgg cctgcggcg atggtgccc ccatgacct caacaagggt  
tgcggtcgg gctgaaggc cgtgatgtg gccgccaacg cgatcatggc gggcgacgce  
gagatcgtg tggccgggg ccaggaaac atgagcgtg ccccgacgt gctgcggggc  
tcgcgcgatg gtttccgat gggcgatgcc aagctggteg acaccatgat cgtcgacggc  
ctgtgggacg tgtacaacca gtaccacatg ggcacacccg ccgagaacgt ggccaaggaa  
tacggcatca caccgcggc gcaggatgag ttcgcgtcg gctcgcagaa caaggccgaa  
gccgcgcga aggcggcga gttgacgaa gagatcgtc ttgtgtgat cccgcagcgc  
aaggcgacg cgggtggcct caagaccgac gagttcgtg gccaggcgc cagctggac  
agcatgtccg gectcaagcc cgccttcgac aaggccggca cggtgaccgc ggccaacgce  
tcgggcctga accgcggcgc cgcgcgggtg gtggtgatgt cggcgccaa ggccaaggaa  
ctgggcctga ccccgctggc cagcatcaag agctatgcca acgcgggtgt cgatcccaag  
gtgatgggca tgggcccggg gccggcctcc aagcgcgcc tgtcgcgcgc cgagtggacc  
ccgcaagacc tggacctgat agagatcaac gaggcctttg ccgcgcaggc gctggcggtg  
eaccagcaga tgggctggga cactccaag gtcaatgtga acggcggcgc catcgccatc  
ggccaccgca tcggcgctc gggctgctg atcctggtga cgctgtgca cgagatgaag  
cgccgtgacg cgaagaagg cctggcctcg ctgtgcatcg gccggcgcat gggcggtggc  
ctggcagtcg agcgcaata a

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Continued on Page 17

Read and Understood By

Lisa L. Mullison10/2

Date

AB FUSION CHARACTERIZATION

noticed I had left the MBx240 pTrc AB fusion on the bench for approximately a week.

MBx240 pTrc AB # 10, 11, 12, 13

LB Amp clear / tan no granules

LB Amp white granules  
1% glucose

LB Amp white granules  
1% glucose  
0.1mM IPTG

eye

light microscopy

start LB glucose O/N culture from freezer stock.

50% x = 1% 100mM

2hr + 0.5hr

1/12-16-97 dilute cultures 1:100 into LB 1% glucose Amp

O/N

@ time dil

4 hrs post -

3hr after inoc.

induction

	O/N	@ time dil	4 hrs post -	induction
pTrc-AB 10	0.46335	4.63	0.7839	1.344
11	0.55087	5.51	0.7959	1.324
12	0.42090	4.21	0.7931	1.513
13	0.50844	5.84	0.7476	1.268

induced

⊕

⊖

5mM IPTG

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take 300ul

samples → 4°C

Continued on Page

92

Read and Understood By

Lara Anderson

Signed

Date

Signed

Date

Cont. from p. 88

LOOK @ CULTURES UNDER MICROSCOPE.

DID NOT HAVE TIME WHEN I TOOK  
SAMPLES FOR WESTERN.

THEREFORE SAMPLES HAVE BEEN @ RT FROM 5 PM  $\rightarrow$  11:15 AM  
\* SAMPLES ARE IN LB 1% GLUCOSE \*

PTC LAB #10

+ IPTG  
granules  
look like peas in  
a pod

-  
single granules @ poles

ooooo

very  
small

#11

"

"

#12

"

"

#13

"

"

$\rightarrow$  many cells w/ inclusion bodies

Majority of cells in presence 5mM IPTG  
are 2-3x ~~more~~ normal length  
while the majority of cells without IPTG  
are 1x cell lengths.

Of course there is always a small majority  
that are 5-10x cell length but only in  
IPTG samples

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Continued on Page

94

Read and Understood By

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Date

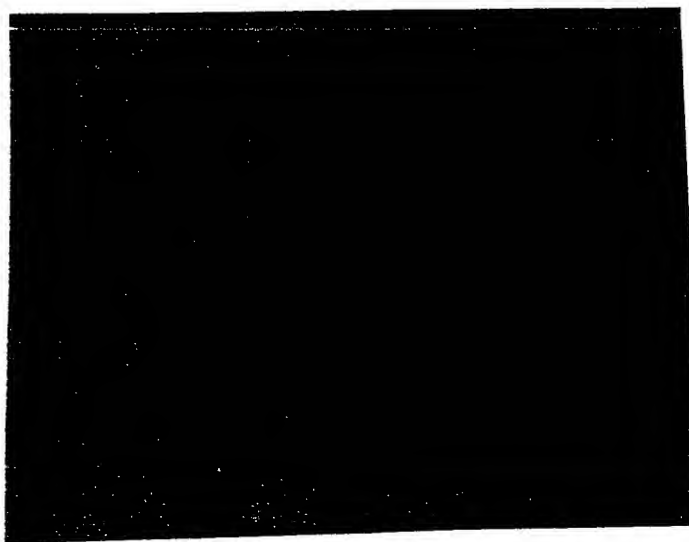
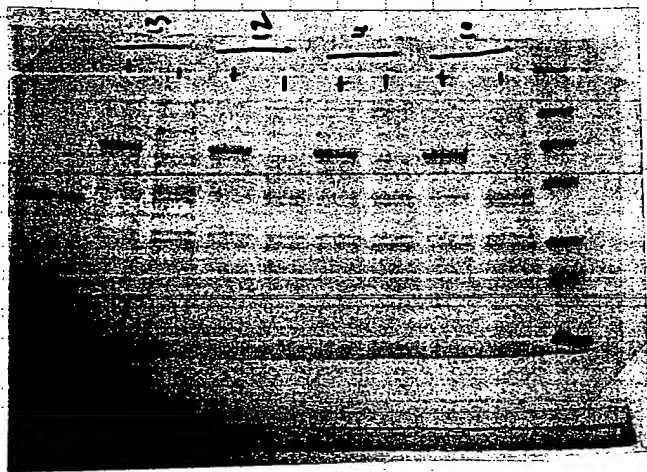
GEL &amp; WESTERN OF AB FUSIONS! p. 88

Lane	Sample	5 mM IPTG	OD <sub>600</sub>	ml for 200	due
(11) 1	MW				
(12) 2	pTrc AB #10	-	1.020	19.6	10
(13) 3		+	1.344	14.9	
(14) 4	pTrc AB #11	-	1.003	20.0	
(15) 5		+	1.324	15.1	
(16) 6	pTrc AB #12	-	0.9694	20.6	
(17) 7		+	1.513	13.2	
(18) 8	pTrc AB #13	-	0.9897	20.2	
(19) 9		+	1.268	15.7	
(20) 10					

$$\frac{200 \text{ (19 ml)}}{\text{ml}} = 1.020 \times$$

19.0 = 27.15 ml bottles  
 10 = 2 x 5 ml brown cap  
 2.12 = 10 ml dye

2 gels → 1 coomassie  
 1 western



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Continued on Page 47

Read and Understood By

Lucia L. Madson

Signed

Date

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